DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Memorandum

Food and Drug Administration
Center for Biologics Evaluation and Research
Bethesda, MD 20892

PRODUCT REVIEW

Date	:	6/15/98
To	:	File
From	:	Steven Kozlowski, Laboratory of Molecular and Developmental Immunology, Division of Monoclonal Antibodies - HFM 561
Through	:	Dr. Kathryn E. Stein, Chief, Laboratory of Molecular and Developmental Immunology, Director, Division of Monoclonal Antibodies K.S. Stein
Subject	:	BLA 97-1359

Therapeutic Agent(s): Synagis™ (Palivizumab), a IgG1,κ humanized antibody against the A epitope of the Respiratory Syncial Virus (RSV) Fusion protein (F-protein). MedImmune code MEDI-493

Sponsor(s): MedImmune

Clinical Indications(s): Synagis™ (Palivizumab) is indicated for the prevention of serious lower respiratory tract disease, caused by RSV, in infants and children with Bronchopulmonary Dysplasia (BPD) or a history of premature birth (< 35 weeks gestation) who are under 24 months of age at the time of first administration.

Product Rationale:
RSV is a major pathogen in infants causing the majority of lower respiratory tract infections in that age group. Infants at highest risk for severe RSV infections include those with premature birth, bronchopulmonary dysplasia, and congenital heart disease. There is no effective vaccine at this time and the available anti viral therapy is limited. Pre clinical trials suggest passive immunization with a monoclonal antibody reactive with the RSV F-protein is an effective strategy in reducing RSV viral titers. Clinical trials with RSVIG-IV have shown that passive immunization with RSVIG-IV is associated with reduced risk of hospitalization for RSV disease in high risk populations. A monoclonal antibody to a conserved epitope (A) of the RSV F-protein is likely to have similar effects. The antibody was humanized in order to lower its immunogenicity and allow repeated dosing during RSV seasons.

Items Reviewed Include:
BLA 97-1359 volumes 1-28 and 96-98 (CBER DLS#L97022528)

BLA submissions: CBER DLS#L98009641, L98004944, L98001300, L98005253, L98007562, L98009080, L98007026, L98002870, L98007526, L98010206, L98010422, L98010264, L98010273
Telecoms: 2/18/98, 3/3/98, 4/21/98, 5/22/98, 6/5/98, 6/9/98
BLA submission dated 6/9/98 and other cited materials
Manufacturing information cbtained at the GMDF inspection 3/26-4/6/98

Notes:
Items written in normal font are taken from BLA submission.
Items in italics are summaries or source locations from BLA material.
Items in bold italic are reviewer comments.

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Background

RSV Clinical Illness

From the clinical review by Dr. Rieves:

RSV, an enveloped RNA virus of the paramyxovirus family, is a common human respiratory pathogen and is the major cause of respiratory tract illness in children.[1] RSV infection is generally acquired via particle inoculation of the nasal or lachrymal mucosa from large droplets (fomites or contact with contaminated secretions. Most RSV infections manifest as respiratory tract disease. Asymptomatic infection is uncommon. Severe RSV disease usually manifests as bronchiolitis or pneumonia and is primarily a disease of infants or immunosuppressed individuals.[2] Infants at greatest risk for RSV include those with a history of prematurity, immunodeficiency, congenital heart disease or chronic lung disease. It is estimated that 50-70% of all infants experience RSV infection in the first year of life and almost all are infected by two years of age. RSV causes more than 90,000 hospitalizations and 4,500 deaths in the United States annually.[3] It is estimated that several hundred thousand infants are at risk for severe RSV infection, although severe respiratory disease will develop in less than one percent of these infants.[4]

Other Prophylaxis and Treatment Options

From reference in footnote 10 (BLA vol 1 pg 31-2):

Treatment options for severe RSV are limited. They include supportive care and the antiviral drug ribavirin. Ribavirin is effective in modifying the course of severe RSV pneumonia and bronchiolitis in immunocompetent individuals, but it requires prolonged aerosol administration and is a potential risk for pregnant women who are exposed to it. Thus its use has been limited.

A formalin inactivated RSV vaccine for prophylaxis has caused more severe RSV disease. This has led to safety concerns regarding other vaccine strategies. In addition primary RSV infection and disease do not protect against subsequent infections. It is therefore unclear whether any of the vaccines under development will lead to effective prophylaxix in the short term. Passive immunization with Respigam™ is currently the only licensed prophylaxis for RSV disease. Respigam™ , a pooled hyperimmune IVIG against RSV has the disadvantage of requiring large volume IV infusions and the potential infectious risks of being manufactured from a pooled human blood product. A monoclonal antibody (MAb) based passive immunization strategy could avoid these problems.

Scientific Rationale

From the BLA section 3.1.1(vol 1 pg 12):

Numerous reports in the literature indicate that MAbs directed to the two RSV surface glycoproteins, the F and G glycoproteins, could prevent or treat RSV infections in mice or cotton rats.[5] While MAbs to both these

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[1] Walsh, E. et al. J Infec Dis. 1997, 175:814-20.

[2] The PREVENT Study Group. Pediatrics 1997, 99:93-99.

[3] Hall, C. Respiratory syncitial virus. Contemp Pediatr. November 1993,:2-11

[4] Committee on Infectious Diseases, Committee on Fetus and Newborn. Pediatrics 1997, 4:645-50.

[5] Taylor G, Stott EJ, Bew M, et al. Lancet 1983:976; Walsh EE, Schlesinger J, Brandriss MW. Infect and Immunity 1984, 43:756-758; Taylor G, Stott EJ, Bew M, et al. Immunology 1984,52:137-142; Walsh EE, Hall CB, Schlesinger JJ, et al. J

proteins were shown to neutralize RSV in vitro and to protect small animals against RSV challenge, antibodies directed to the F protein are likely to be preferable for clinical use. There are two antigenically distinct subtypes of RSV, designated A and B. Although antigenic and structural differences between A and B subtypes have been described for both the F and G proteins, the more significant antigenic differences reside on the G glycoprotein where amino acid sequences are only 53% homologous and antigenic relatedness is 5%.[6] In addition, monoclonal antibodies raised to the G glycoprotein are usually subtype specific.[7] Conversely, antibodies raised to the F protein show a high degree of cross-reactivity among subtype A and B viruses.

Beeler and Coelingh[8] conducted an extensive analysis of 18 different murine monoclonal antibodies directed to the RSV F protein. Comparison of the biological and biochemical properties of these MAbs resulted in the identification of three distinct antigenic sites (designated A, B and C). Neutralization studies were performed against a panel of RSV strains isolated from 1956 to 1985 which demonstrated that epitopes within antigenic sites A and C are highly conserved while the epitopes of antigenic site B are variable. Thus to obtain the broadest coverage of clinical virus isolates, a MAb directed to either the A or C site, should be chosen.

The clinical use of murine MAbs to prevent RSV infections in humans would be hampered by induction of human anti-mouse antibody (HAMA) responses. By substituting the mouse complementary determining regions (CDRs) into human variable framework segments, it is expected that HAMA responses would be minimized, and the binding affinities and specificities to the RSV F protein should be retained. Since the CDRs do not contain uniquely murine or human motifs, human antibodies containing the murine antibody CDRs should be essentially indistinguishable from completely human antibodies. Tempest et al. , applied this CDR grafting technique to a murine MAb specific for a linear epitope of the F protein of RSV.[9] This "humanized" MAb recognized all 24 clinical isolates of RSV tested (representing subgroup A and B strains), neutralized RSV in vitro, inhibited virus-induced cell fusion, and prevented or resolved RSV infections in mice as effectively as the original mouse MAb.

MedImmune has developed MEDI-493, a CDR-grafted humanized antibody derived from MAb 1129, a murine MAb described by Beeler and Coelingh [8], which is directed to the antigenic site A on the RSV F protein. This antibody is highly effective in neutralizing a large panel of contemporary clinical isolates of RSV, inhibiting virus-induced cell fusion and protecting cotton rats against RSV infection.[10] Based on the potency of this molecule, it is expected that protective serum and tissue levels of neutralizing antibody can be achieved with a small injection volume, possibly permitting subcutaneous or intramuscular administration as well as intravenous infusion.

<u>Biologic Activity</u>

From BLA section 3.6.1 (vol 1 pg 113) and 7 (vol 28 pg 1), and the references in footnotes 10(vol 1 pg 16-25) and 8 (vol 1 pg 31-40):

The humanized antibody MEDI-493 or Palivizumab has been evaluated in various preclinical studies. Plasmon resonance studies have shown a Kd of 1.4

Gen Virol 1989,70:2953-2961; Taylor G, Furze J, Tempest, PR, et al. Lancet 1991,337:1411-2; Taylor G, Stott EJ, Furze J, et al. J Gen Virology 1992,73:2217-2223; Crowe J Jr, Murphy BR, Chanock RM, et al. Proc Natl Acad Sci 1994, 91:1386-90.

[6] Walsh EE, Hall CB, Briselli M, et al. J Infect Dis 1987,155:1198-204; Johnson PR, Spriggs MK, Olmsted RA, et al. Proc Natl Acad Sci 1987,84:5625-5629.

[7] Taylor G, Furze J, Tempest, PR, et al. Lancet 1991,337:1411-2.

[8] Beeler JA, Coelingh K. J Virology 1989, 63:2941-2950.

[9] Tempest PR, Bremner P, Lambert M, et al. Bio/Technology 1991,9:266-271.

[10] Johnson S, Oliver C, Prince G, et al. JID 1997,176:1215-1224.

nM for binding to the RSV F-protein. In a fusion-inhibition assay using the RSV A Long strain the EC_{50} was 0.17 ± 0.6 mcg/ml. Both the microneutralization test (EC_{50} 0.1 ± 0.3 mcg/ml) and the plaque reduction neutralization test (2 mcg/ml)have been used to evaluate Palivizumab *in vitro*. The plaque reduction neutralization test results were similar with the RSV A Long strain or the RSV B 18537 strain. In the microneutralization assay, Palivizumab neutralized 34 RSV subtype A and 23 RSV subtype B clinical isolates. A resistant virus preparation of RSV was generated by passaging the virus in the presence of Palivizumab This resistant RSV was neutralized by RSV-IVIG but not by Palivizumab [10](BLA vol 1 pg 34). Other MARMs (monoclonal antibody resistant mutants) of RSV have been generated by selection in anti F-protein monoclonal antibodies.[8] These mutants often are resistant to multiple monoclonal antibodies and can be selected at a frequency of 10^{-4} to 10^{-6}. Some of these MARMs display reduced plaque size but the MARM induced by the murine parent antibody of Palivizumab, MAb 1129, had a normal plaque size. In order to test for *in vivo* selection of antibody resistant mutants, lung homogenates of cotton rats prophylaxed with varying doses of Palivizumab and infected with RSV were combined with 0.1 mg/ml Palivizumab and added to HEp-2 cells. Out of the 30 Palivizumab animals used in the experiment, only one RSV plaque was recovered and that was shown to be sensitive to Palivizumab in both plaque reduction and microneutralization assays (BLA section 7.2.4 vol 28 pg 34).

In prophylaxis studies in the cotton rat model, Palivizumab levels of 25-30 mcg/ml reduced the mean pulmonary RSV titer by 99%. All animals with levels of 40 mcg/ml achieved this level of pulmonary RSV titer reduction. This effect was seen with IV or IM treatment. Cotton rat studies did not show enhancement of pathology with Palivizumab prophylaxis followed by RSV infection (Table 7.2.4-2, BLA vol 28 pg 36). To evalute the secondary response of cotton rats prophylaxed with Palivizumab and infected with RSV, these rats were given a second RSV challenge when their Palivizumab levels dropped 0.01 ± 0.01 mcg/ml, at 8 weeks post challenge (BLA section 7.2.5, vol 28 pg 37-9). The treated mice were resistant to the second RSV challenge having undetectable viral titers in the lungs and no lung pathology attributable to RSV. Control mice had low lung viral titers and 1/8 had lung pathology attributable to RSV. This is consistent with the low titer of the second challenge (10^{3} pfu/animal). Palivizumab PK was also shown to be unaffected by an RSV infection.

Two studies in cynomolgus monkeys demonstrated a distribution time or $t_{1/2}\ \alpha$ of 0.24-30.66 hours, and a half life or $t_{1/2}\ \beta$ of 4.2-10.7 days. Only one animal developed a low level immunoreactivity to Palivizumab. No changes were observed in blood chemistries, urinalysis or coagulation profiles and no microscopic findings were attributable to Palivizumab. A study in rabbits to assess IM and subcutaneous routes of administration, with the reconstituted lyophilized product, revealed no lesions attributable to Palivizumab at the injection site on days four and fourteen. A high dose toxicity study in rats was performed with Palivizumab doses of up to 840 mg/kg. Again, no changes were observed in blood chemistries, urinalysis or coagulation profiles and no microscopic findings were attributable to Palivizumab. A tissue cross reactivity study was performed with adult and neonatal tissues. No cross reactivity was observed (BLA vol 28 pg 2-3).

Production Facility Overview

This is taken from section 3.4.1 of the BLA vol 1 pg 84-88 and Form FDA 356h:

Manufacturing Locations

Palivizumab or MEDI-493 is manufactured by MedImmune, Inc. in the Licensable Manufacturing Area (LMA) of the Gaithersburg Manufacturing and Development Facility (GMDF) located at 35 West Watkins Mill Road in Gaithersburg, Maryland, USA. Quality Control (QC) testing is performed by MedImmune, Inc. at this site and the Frederick Manufacturing Center (FMC)located at 636 Research Drive in Frederick, Maryland, USA. Filling, lyophilization, labeling and packaging will occur

Gaithersburg Manufacturing and Development Facility

The GMDF is a multi-product facility designed and constructed to support the manufacturing of preclinical, clinical, and licensable products. The GMDF consists of five areas: the Licensable Manufacturing Area (LMA), the Development Manufacturing Area (DMA), the Support Area, the Common Area, and the Mechanical/Receiving Area. The floor diagram of the GMDF is shown in Figure 3.4.1-1. Detailed equipment descriptions and floor diagrams for each area are provided in the Establishment Description Sections 15.2 to 15.5. MEDI-493 cell culture and purification operations are performed in the LMA which consists of the following areas: Cell Bank Storage Room, Inoculum Expansion Room, Media Preparation Room, Bioreactor Room, Isolation Room, Buffer Preparation Room, Clean Storage Room, and a 4-room Purification Area. The scale of MEDI-493 production Other products are manufactured in the DMA which consists of a Cell Culture Area, a Purification Area, and a Fermentation Room. The LMA and DMA share the Support Area which provides the services of glassware wash, autoclave, depyrogenation oven, tank wash, clean-in-place (CIP), and storage/weighing of raw materials. The Support Area consists of the following areas: Glassware/Tank Wash Room, Dirty Storage Room, CIP Room, Weigh Room, Raw Material Ambient Storage Room, Raw Material Cold Storage Room, Product Storage Cold Room, and DMA Clean Storage Room. The Common Area consists of the following sections: Change Rooms, Rest Rooms, Break Room, Computer Room, and Quality Control (QC) Laboratory. The QC Laboratory supports the environmental monitoring program, utility testing, raw material release, in-process testing, drug substance and drug product release, and stability testing. The Mechanical/Receiving Area contains the utility systems which include plant steam, reverse osmosis deinonized (RODI) water, clean steam, water for injection (WFI), compressed air, process gases (oxygen, nitrogen, and carbon dioxide), and waste treatment. The Mechanical/ Receiving Area also contains the Metrology Laboratory, the Gowning Storage Room, and the Loading Dock.

Access to the GMDF is limited to authorized personnel by programmable card key. Upon entering the facility, personnel change into plant uniforms and plant shoes. Upon entering the production areas (LMA and DMA), personnel put on hair covers, face masks, gloves, sterile gowns, and shoe covers. The rooms in the production areas are constructed with surfaces that are smooth and easy to clean. Light fixtures are recessed and sealed. Cleaning and sanitization procedures are performed daily, weeklv. and quarterly according to approved SOPs. The LMA-Purification Area is , while the LMA-Cell Culture Area and the Support Area are The temperature, humidity, and pressure in each room are controlled and recorded. The classified areas are routinely monitored for air quality and surface bioburden. This includes measurement of viable and non-viable air particles, measurement of room and equipment bioburden, and identification of viable contaminants. In addition, routine monitoring is performed on water, clean steam, and compressed air used in the GMDF.

The calibration and validation programs encompass the facility, equipment, process operations, and analytical test methods (Sections 4.2.4.2, 6.0, 15.7 and 15.8). Major utilities (e.g., HVAC, RODI water, clean steam, WFI, compressed air, CIP); facility equipment (e.g., glass washer, autoclave, depyrogenation oven); and process-related equipment (e.g., bioreactors, chromatography controller, laminar flow cabinets, cold storage areas, tanks, pressure cans) have been validated. Performance qualifications for process solution and product contact equipment include validation of the cleaning procedures. Although the GMDF is a multi-product facility, there are no other cell lines or products manufactured or handled in the LMA. Production equipment (e.g., glassware, bioreactors, columns, tanks) and personnel are dedicated to the MEDI-493 process. Shared areas include those for buffer preparation, glassware/tank cleaning, and storage/dispensing of raw materials. In these areas, MEDI-493 activities are separated from activities for other products by time and procedure (Section 4.2.2.2). Control, oversight, training, and scheduling of activities in the shared areas is the shared responsibility of the LMA, QC, and/or Materials Management staff.

Quality Control Laboratories at the GMDF

The quality control laboratory in the GMDF supports the environmental monitoring program, utility testing, raw material release, in-process testing, and drug substance and drug product lot release and stability testing. HPLC and spectrophotometric methods used for in-process and product testing are carried out in Analytical test methods requiring the use of radioisotopes and the fluorescence spectrophotometer are performed by Quality Control analysts in of the Gaithersburg Facility. Some of the equipment for these tests are located in the laboratory equipment corridor,

Quality Control Laboratories at the Frederick Manufacturing Center

There are four QC laboratories at the FMC: Chemistry, Plasma Screening, Microbiology, and Product Testing. The layout of the laboratories is illustrated in Figure 3.4.1-2. The Microbiology Laboratory performs testing to support the environmental monitoring program, utilities monitoring program, and in-process control. These tests include bioburden, endotoxin analysis, and microbial identification. The Chemistry Laboratory supports raw material release, in-process, final product release, and stability testing. This includes HPLC and spectrophotometric assays. The Product Testing Laboratory is responsible for in-process, drug substance and drug product release, and stability testing. This includes electrophoretic and immunologic assays. The Screening Laboratory, a laboratory unrelated to MEDI-493, is responsible for testing incoming plasma samples for product specific titers.

Structural Characterization

MEDI-493 is characterized using methods that measure the physical and chemical properties of the molecule (for an overall review, see Davis et al., 1994). The characterization/proof of structure was determined from data supporting the primary, secondary, and tertiary structure of MEDI-493. A brief description of the test methods is found in the Appendix Section 4.2.1.2.1.1.1. Table 4.2.1.2.1-1 summarizes the biochemical methods used to elucidate the protein structure of the MEDI-493 product and is shown below:

Table 4.2.1.2.1-1 Biochemical Tests for Structural Analysis

Test	Structural level	Use
Amino acid analysis	Primary	Verify amino acid content compared to that predicted by the cDNA
N-terminal sequencing	Primary	Verify the integrity of the N-terminus
In situ CNBr fingerprint sequencing	Primary	Verify the integrity of selected internal sequences
UV spectrum	Primary	Verify that spectral profile is consistent with a protein
IgG isotype	Primary/secondary/tertiary	Confirm IgG subclass
Peptide mapping with MALDI-TOF MS* or LC-ESMS**	Primary	Confirm primary structure and posttranslational modifications
Monosaccharide composition analysis	Primary	Determine carbohydrate content
Oligosaccharide profile analysis	Primary	Distinguish and identify oligosaccharide heterogeneity
SDS-PAGE (non-reducing)	Tertiary	Determine purity and identify aggregates
SDS-PAGE (reducing)	Primary	Determine purity by detecting breakdown products
Reducing isoelectric focusing	Primary	Determine charge heterogeneity
Size-exclusion chromatography	Tertiary	Determine the presence of aggregates or breakdown products
Differential scanning calorimetry	Tertiary	Verify consistent thermal stability profile
MALDI-TOF mass spectrometry	Tertiary	Verify molecular weight of intact IgG, identify impurities by mass
Capillary gel electrophoresis (CGE)	Primary/tertiary	Determine purity by detecting breakdown products
Capillary isoelectric focusing (CIEF)	Primary	Determine charge heterogeneity
Laser light scattering	Tertiary	Identify aggregates and breakdown products

*Matrix-assisted laser desorption time of flight mass spectrometry

**Liquid chromatography electrospray mass spectrometry

BLA Section 4.2.1.2.1 contains data suggesting that the primary, secondary and tertiary structure of MEDI-493 are as expected

MEDI-493 is a glycoprotein with about 1-2% carbohydrate (by weight as determined by GC-MS analysis) attached to a single site on the heavy chain (Asn-300) in an N-linkage (determined by sequencing and LC-ESMS). The carbohydrates attached to the heavy chain appear to be predominantly either in a biantennary fucosylated complex-type which may be terminally galactosylated or a high mannose form. *(Some of the deduced oligosaccharide structures are shown in the BLA Figures 4.2.1.2.1-18 and 4.2.1.2.1-19.)* This is the typical site for N-glycosylation on humanized monoclonal IgGs and was confirmed by sequencing and LC-ESMS. Confirmation of these structures on MEDI-493, was provided through monosaccharide composition analysis and oligosaccharide profile analysis.

Several techniques were used to assess the secondary and tertiary structure of MEDI-493. These techniques included: differential scanning calorimetry (DSC), laser light scattering, and peptide mapping. DSC was used to determine the temperature at which thermal transitions (i.e., denaturation) may occur to the protein. Laser light scattering was used to confirm the molecular weight of the native MEDI-493 molecule as well as a purified aggregate molecule, and peptide mapping was used to confirm the location of disulfide bonds between polypeptide chains.

Impurity types can be classified as either related to or derived from the product (e.g., aggregates, breakdown products), or unrelated to MEDI-493 (e.g, host cell proteins, medium components). Unrelated impurities are discussed in BLA Section 4.2.6.3. Various product-related impurities may be found in monoclonal antibody preparations as a result of degradation or modifications. These impurities include aggregated, deamidated, oxidized, truncated at the C-terminus, and fragmented forms. A variety of techniques were used to characterize MEDI-493 product-related. A small amount of higher order aggregate (approximately 1 million Da) was seen in the laser light scattering analysis. In addition, a 92 kDa polypeptide is detected in MEDI-493 on reducing SDS-PAGE gels which is both heavy and light chain immunoreactive. Based on the size and immunoreactivity of the polypeptide, it is likely that this is a covalently linked single heavy and two light chains. MEDI-493 does not have any detectable levels of oxidized Met residues but oxidation of Met residues in MEDI 493 can occur. Truncation of the heavy chain C-terminal Lys has been observed in recombinant IgGs produced in cell culture. In MEDI 493, the ratio of truncated C-terminal fragments to the complete C-terminal fragment varies from 2.3 for lot L97A043 to 4.0 for lot L96G025. Deamidated proteins at Asn or Gln residues are commonly found in monoclonal antibodies. MEDI-493 was reduced, subjected to trypsin digestion, and the fragments separated by reversed-phase HPLC in order to detect any deamidation products in the native molecule. The separated fragments were analyzed using both UV detection and ESMS (Appendix Section 4.2.1.2.1.1.8). The results indicate that deamidation products are observed in MEDI-493 in the form of isoaspartic acid and aspartic acid. MEDI-493 was also exposed to pH 10 conditions for 15 days at 45 °C in order to accelerate the rate of deamidation. Following treatment at high pH, deamidation primarily occurred at two locations on the heavy chain (Asn-387 and Asn-392).

Host Cell and Expression Vector System

Taken from BLA Process Overview section 3.4.2 vol 1 pg 89-107:

The host cell and expression vector system used for manufacture of MEDI-493 consists of a mouse myeloma host cell (NS0) transfected with a plasmid vector (pMI228) containing expression cassettes for both MEDI-493 heavy and light chain genes (Section 4.2.3.3). The generation of the recombinant cell line expressing MEDI-493 involved a series of steps including: 1) the production and selection of murine monoclonal antibody MAb 1129, 2) the humanization of MAb 1129, and 3)the construction and selection of the expression NS0 cell line. Twenty one candidate production cell lines were evaluated for growth rate and secretion of antibody in T-flasks and spinner flasks. The best candidate cell line was adapted to serum-free medium DMNS0-4 and subsequently subjected to two rounds of single-cell cloning. From this point forward cells were grown in DMNS0-4 medium. The final selected clone was expanded to generate 5 vials preserved in liquid nitrogen as the Accession Cell Bank (ACB).

Production Of Murine Hybridoma Cell Lines

(summarized from BLA section 4.2.3.3.1.1 vol 6 pg 118-21)

MAb-1129 is one of a number of murine hybridoma cell lines developed at the NIAID and CDC and described by Beeler and Coelingh.[8] BALB/c mice were immunized with human RSV strain A2 and boosted with vaccinia virus recombinant expressing RSV F protein or RSV strain A2. Spleens from these mice were fused with NS-1 murine myeloma cells and plated for individual colonies. Colonies which were positive for antibodies against RSV F protein underwent 2 rounds of limiting dilution to obtain 18 hybridoma lines. Ascites was prepared from these hybridoma lines and tested for F-protein binding sites using RSV mutants and competition studies. Three non-overlapping F-protein sites A, B, and C were identified using these antibodies. Antibodies against sites A and C appeared conserved among 23 clinical isolates. Eight of these antibodies were evaluated by MedImmune by intranasal prophylaxis (at 10, 1, 0.1 mg/kg) followed by RSV challenge at 24 hrs. Four days later pulmonary titers were evaluated. MAb-1129 was one of the more effective antibodies, demonstrating a 2-3 log reduction in virus titers. The prophylaxis was also effective when given IM. MAb-1129 was 50-100X more effective than RSV-IGIV in the cotton rat model *(see BLA vol 28 pg 26)*.

<u>The humanization of MAb 1129</u>

(summarized from BLA section 4.2.3.3.2 & 4.2.3.3.3.1 vol 6 pg 122-37)

Messenger RNA from the hybridoma line MAb-1129 was purified and cDNA was generated. The VH and VL genes were amplified from the cDNA by PCR[12] and cloned into the pUC18 vector. The cloned variable regions were sequenced and the predicted amino-acid sequences were compared to known human variable region sequences.[13] The human VL with the greatest homology was K102 with Jκ-4 and the human VH with the greatest homology was with Cor and Cess (closely related VH-II family members). Models, substituting these sequences into the coordinates of the MCPC603 crystal structure crystal, were used for designing the CDR grafted human variable regions.

The CDR grafted VH gene was generated by assembly of synthetic oligonucleotides using PCR).

The humanized VL gene was generated by site directed mutagenesis (Figure 4.2.3.3.2.5-2 vol 6 pg 134)of another CDR grafted VL region

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[12] Orlandi R, Gussow D, Jones P, et al. Proc Natl Acad Sci USA 1989,86:3833-7.

[13] Queen C, Schneider W, Selick H, et al. Proc Natl Acad Sci USA 1989,86:10029-33

The construction and selection of the expression NSO cell line

(summarized from BLA section 4.2.3.3.3.2 & 4.2.3.3.3.3 vol 6 pg 138-41)

NSO cells are glutamine auxotrophs, with an absolute requirement for added glutamine or a glutamine synthetase gene. The use of glutamine deficient media allows for selection of cells transfected with expression vectors containing the glutamine synthetase gene. Then the use of methionine sulphoxamine (MSX), a glutamine synthatase inhibitor allows for the selection of NSO cells with high copy numbers of the expression vector. The increased copy number also leads to increased production of product by the transfected NSO cells.

. The NSO cells were grown in

. One vial was thawed and passaged 4 additional times and then electroporated with

Palivizumab (MEDI-493) expressing plasmid, was linearized witl EtOH precipitated and used for the electroporation. The tranfected NSO cells were selected in glutamine deficient IMDM supplemented with 10% FCS

). 147 colonies were analyzed by ELISA for human antibody. The 7 highest producing colonies (in pg/cell/day) were selected in MSX (a glutamine synthetase blocker) The best of 21 subclones, was adapted to serum free medium and cloned by limiting dilution twice in the serum free medium. From this point forward, cells were grown exclusively in hese limiting

After the second round of cloning, the best cell line was selected. This clone was expanded to generate five vials o ells which were preserved in liquid nitrogen as the Accession Cell Bank (ACB). on

Components of Serum-Free Medium for transfected NSO cells: (Certificates of analysis available for all components; all bovine protein components were certified free from bovine viruses bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), and parainfluenza 3 (PI-3); the components were obtained from US/Canadian suppliers, have traceable histories and the certificates of origin are attached in Appendix 4.2.3.4.4)

(This is from BLA Table 4.2.3.4.1-1)

Component	Source

Cell Banks

Taken from BLA Process Overview section 3.4.2 vol 1 pg 89-107 and section 4.2.3.4, Description of the Cell Seed Lot System:

A vial of cells from the ACB was expanded through a series of T-flasks and spinner flasks to generate a Master Cell Bank (MCB). The MCB was characterized for identity, quality, and safety. No evidence of microbial or viral contamination was found, the species of the cell line was confirmed to be of murine origin, and the DNA profile was characterized. The cDNA sequences for the heavy and light chains were identical to those predicted for MEDI-493 expressed from the vector pMI228, and the copy number and structure of the expression vector were shown to be consistent with the primary transfectant. The MCB was further expanded to generate a Working Cell Bank (WCB). Characterization of the WCB showed no evidence of microbial or viral contamination, and the DNA profile was consistent with that of the MCB. All production lots are initiated from the WCB. The cell line characterization was performed as recommended in FDA's "Points to Consider in Characterization of Cell Lines Used to Produce Biological Products, 1989" and "Points to Consider (PTC) for the Characterization of Cell Lines Used to Produce Biologicals and for the Manufacturing and Testing of Monoclonal Antibody for Human Use, 1997," and the ICH guideline "Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human and Animal Origin, 1996." The preparation and characterization of the cell banks is described in Section 4.2.3.4. of the BLA.

MCB Generation

Cells from one ACB vial were expanded through a series of T-flasks and, subsequently, spinner flasks, [redacted] Cells from the spinner flask cultures were centrifuged and resuspended in a serum-free freezing medium to establish a [redacted] MCB [redacted]. The [redacted]. Each MCB vial can be used to prepare a Working Cell Bank (WCB). Each WCB can support approximately 15 years of production [redacted] This yields a total of approximately 3000 years of production capability.

MCB Characterization

(from BLA Table 4.2.3.4.1-2)

Mouse Antibody Production	Negative; no evidence of viral contamination
Cell Line Species Identity by Isoenzyme Analysis	Consistent with a murine cell line
In Vivo Assay for Adventitious Viruses	Negative; no evidence of viral contamination
In Vitro Assay for Adventitious Viruses	Negative; no evidence of viral contamination
Sterility	Negative; no evidence of microbial contamination
Mycoplasma	Negative; no evidence of mycoplasma contamination
Extended S+L- Focus Assay	Negative; no evidence of viral contamination
Extended XC Plaque Assay	Negative; no evidence of viral contamination
DNA profile	DNA profile characterized cDNA Sequences of H & L Chains Identical to the predicted sequences Copy Number 3 copies of the plasmid/cell Structure of Expression Vector Consistent with the primary transfectant

WCB Generation

Cells in one MCB vial were expanded through a series of T-flasks and, subsequently, spinner flasks, [redacted] was achieved. Cells from the spinner flask cultures were centrifuged and resuspended in a serum-free freezing medium to establish a [redacted] WCB [redacted]

WCB Characterization

(from BLA Table 4.2.3.4.2-1)

Sterility	Negative; no evidence of microbial contamination
Mycoplasma	Negative; no evidence of mycoplasma contamination
DNA Profile	DNA profile consistent with MCB profile

End of Production Cells (EPC)

The End of Production cells (EPC) were collected aseptically at the end of the cell culture production process from the production bioreactor. The EPC were characterized for identity, quality, safety, and stability. The characterization was performed as recommended in FDA's "Points to Consider in Characterization of Cell Lines Used to Produce Biological Products, 1989" and "Points to Consider (PTC) for the Characterization of Cell Lines Used to Produce Biologicals and for the Manufacturing and Testing of Monoclonal Antibody for Human Use, 1997," and the ICH guideline "Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human and Animal Origin, 1996." No evidence of viral contamination was found in the mouse antibody production (MAP) test, in vivo assay, and in vitro assay for adventitious viruses. No evidence of microbial mycoplasma contamination was found in the sterility and mycoplasma tests. The negative stain electron microscopy results indicated that the concentrations of the Type A and Type C retroviral particles in the EPC samples ranged from particles/mL.

The EPC samples containing these retroviral particles were further tested by the S+L- focus forming and XC plaque assays. No evidence of retroviral contamination was detected. In addition, the EPC samples were also characterized by a co-cultivation assay utilizing a human rhabdomyosarcoma and a mink lung cell line. Endpoint S+L- focus forming assay and the reverse transcriptase assay confirmed the absence of infectious retroviral particles in both human and mink cell lines. The species of the cell line was confirmed to be of murine origin.

The sequences of cDNAs for the heavy and the light chains obtained from the EPC were determined by direct sequencing of PCR amplified first strand cDNA using an automated DNA sequencer and dye labeled dideoxynucleotide terminators. The sequences obtained were identical to those predicted for MEDI-493 as expressed from the vector The copy number and structure of the expression vector were verified to be identical to those of the MCB.

The frequencies of the EPC tests are as follows. The sterility, mycoplasma, and in vitro adventitious viruses assays are performed on all production lots. Quantitation of viral contaminants by the negative stain electron microscopy is performed on the three consistency lots while the rest of the tests are performed on a representative lot only. A similar testing schedule will be observed when the production scale increases.

Characterization of MEDI-493 End of Production Cells

(from BLA Table 4.2.3.4.3-1)

Test	Frequency			
Mouse Antibody Production (MAP)	1 lot	ND	Negative	ND
Cell Line Species Identity by Isoenzyme Analysis	1 lot	ND	Consistent with a murine cell line [4]	ND
In Vivo Assay for Adventitious Viruses	1 lot	ND	Negative; no evidence of viral contamination	ND
In Vitro Assay for Adventitious Viruses	All lots	Negative: No evidence of viral contamination	Negative: No evidence of viral contamination	Negative: No evidence of viral contamination
Sterility	All lots	Negative: No evidence of microbial contamination	Negative: No evidence of microbial contamination	Negative: No evidence of microbial contamination
Mycoplasma	All lots	Negative; no evidence of mycoplasma contamination	Negative; no evidence of mycoplasma contamination	Negative; no evidence of mycoplasma contamination
Amplified PG4 S+L-Focus Assay for Retroviruses	1 lot	Negative: No evidence of viral contamination	ND	ND
Extended XC Plaque Assay for Retroviruses	1 lot	Negative: No evidence of viral contamination	ND	ND
Co-Cultivation Assay with Human Rhabdo-myosarcoma and Mink Lung Cells	1 lot	ND	Negative: No evidence of viral contamination	ND
Negative Stain Electron Microscopy	3 lots	type particles/ml	type particles/ml	type particles/ml
cDNA Sequence H & L chains	1 lot	ND	Identical to the predicted sequences	ND
Copy Number	1 lot	ND	Consistent with the MCB [5]	ND
Structure of Expression Vector	1 lot	ND	Consistent with the MCB	ND

ND: Not determined

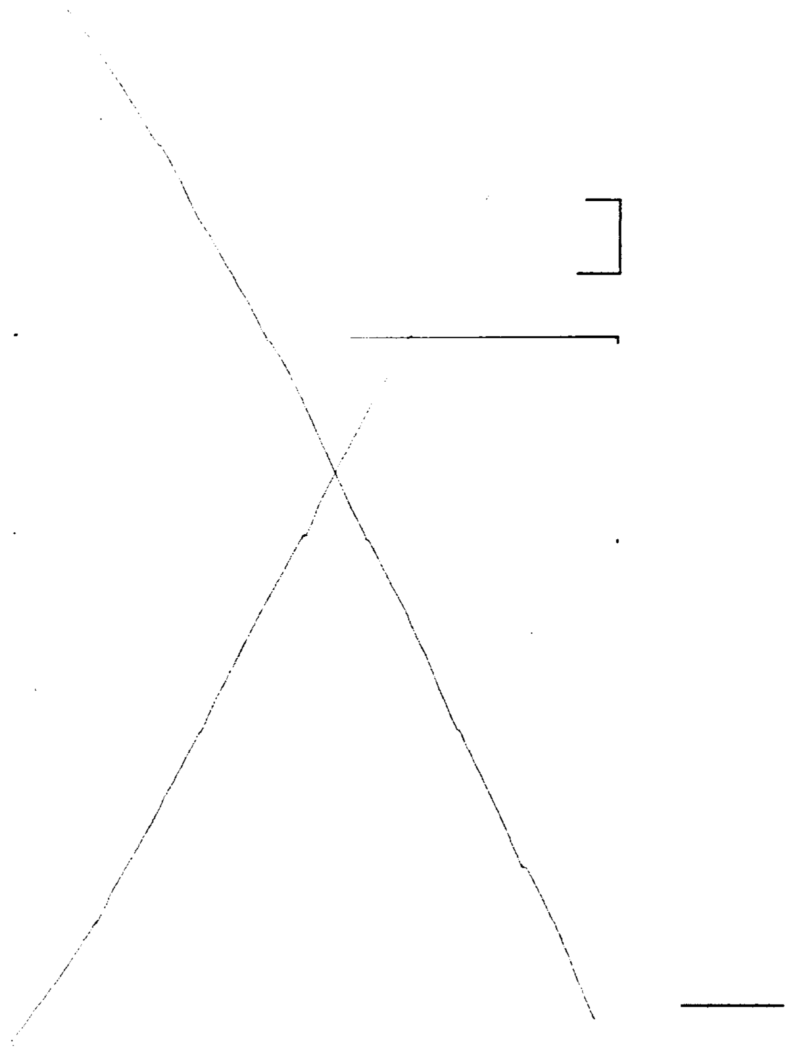
The Extended Cell Bank (ECB) and Production Cell Line Stability

As the scale of the cell culture process is increased, cells of higher population doublings (PDLs) are used in the production bioreactor. Several studies were performed to examine the stability of the cell line at increasing PDLs. An initial study showed consistent cell growth, antibody production, and doubling time over a 6 month period which represented 53 serial passages (75 PDLs) in serum-free medium . To further examine cell line stability an extended cell bank (ECB) was established by passing cells in DMNSO-4 medium through 47 PDLs beyond the WCB. The estimated numbers of cell doubling required for a commercial production process is approximately 28 PDLs from the WCB. Characterization of the ECB (PDL=75) confirmed the cells to be of murine origin, and the DNA profile, growth, and productivity were consistent with those of the WCB (PDL=28) and MCB (PDL=20

Cell Fermentation

Taken from BLA Process Overview section 3.4.2 vol 1 and section 4.2.3.5, Description of the Cell Growth and Harvesting Process:

MEDI-493 is produced in serum-free medium using a stirred-tank fed-batch process (*BLA Section 4.2.3.5*). A vial of cells from the WCB is expanded through a series of T-flasks and spinner flasks. From the spinner flasks, the cells are pooled and inoculated into a (total volume) expansion bioreactor. The culture is transferred into a (total volume) production bioreactor for the final stage of cell culture production where glucose and nutrient feeds are supplied to the culture. The cells and debris are removed from the culture using tangential-flow microfiltration, and the pH and conductivity of the resulting cell-free conditioned medium are adjusted for further processing. End of Production Cells (EPC) are collected and tested following every production run (*BLA Section 4.2.3.4.3*). EPC testing for clinical and consistency lots (*as described above*) has revealed: no evidence of microbial contamination to date; that the species of the cell line is of murine origin; that the cDNA sequences for the heavy and light chains are identical to those predicted for MEDI-493 expressed from the vector and that the copy number and structure of the expression vector are consistent with the MCB. *BLA Figure 4.2.3.2-1 describes the process:*



Fermentation Media

MedImmune proprietary serum-free culture medium

. A nutrient feed, which contains amino acids and vitamins, is used in bioreactor cultures to sustain the cell growth and antibody production. The composition of the nutrient feed is listed in BLA Table 4.2.3.5-2.

Components of Serum-Free Medium

[1] Certificates of analysis available for all components; certificates of origin of the bovine components are included in BLA Section 4.2.3.4.4.

Components of Nutrient Feed

[1] Certificates of analysis are available for all components

Fermentation Equipment

- Biological safety cabinet
- CO_2 incubators
- Water bath
- Microscope
- Magnetic stir plates
- Spinner flasks (100-3000 mL)
- glass transfer bottles
- stirred-tank bioreactor
- stirred-tank bioreactor
- Tangential-flow microfiltration unit
- Peristaltic pumps
- Balances
- Fixed stainless steel tanks
- Portable vessels: glass bottles stainless steel tanks

Fermentation Process

A vial of cells from the WCB is thawed at 37±2 degrees C in a water bath and resuspended in approximately 30 mL of medium in a T-150 flask. The flask is placed in a humidified 37±2 degree C incubator with CO_2 concentration controlled at 6±1%. As shown in Figure 4.2.3.2-1 earlier in this section, the culture is expanded in a series of T-flasks and, subsequently, spinner flasks to obtain approximately of cell suspension. At each stage, the cells are inoculated at greater than and expanded to the subsequent stage when the cell concentration reaches . From the spinner flasks, the cells are aseptically pooled into a transfer bottle for the inoculation of an expansion bioreactor (e.g., working volume). An autoclaved antifoam solution , working volume production bioreactor) is aseptically added to the transfer bottle to suppress sparging-induced foaming in the expansion and production bioreactors. Prior to initiation of the expansion bioreactor culture, the bioreactor is cleaned and sterilized and the dissolved oxygen (DO) probe and the pH probe are calibrated.

When the cell concentration in the expansion bioreactor reaches), the culture is transferred

When the cells reach a concentration between (approximately a volume of nutrient feed bioreactor working volume is added into the bioreactor

conditioned medium, which contains MEDI-493, is collected in a harvest tank which has a working volume of 2-3 times the bioreactor working volume (e.g., harvested conditioned medium is stored at 2-8°C for no more than 3 days until it is further processed The acceptance criteria for the conditioned medium intended for further manufacture are: (a) product concentration is greater than nd (b) EPC is free from microbial contaminants. A stability study indicated that the conditioned medium is stable at 2-8°C for at least 4 weeks (Table 4.2.3.5-3) based on the measurements of high performance size exclusion chromatography (HPSEC), high performance ion-exchange chromatography (HPIEC), Protein A HPLC, SDS-PAGE, isoelectric focusing (IEF), and the microneutralization assay.

Aseptic Transfers

All open manipulations are performed in a Class 100 biosafety cabinet (BSC) under laminar flow HEPA-filtered air using aseptic technique The bioreactors and the harvest tank were validated to be closed systems. The vessels are equipped with steam-sterilizable ports for aseptic sampling and addition of inoculum, media, and nutrient feeds. Prior to inoculating the expansion bioreactor, the spinner flask cultures are pooled into an autoclaved transfer bottle in the Class 100 BSC. The transfer bottle is equipped with a dip-tube which is connected to a silicone tubing transfer line at the bottle cap. At the end of the transfer line, a closed diaphragm valve is employed to protect the sterile envelope. The filled transfer bottle is transported on a cart to the expansion bioreactor and the diaphragm valve is connected to the inoculation port with a tri-clamp. The connection is steam sterilized for45±5 minutes. After the line cools down, the diaphragm valve and inoculation valve are opened and the culture is transferred into the expansion bioreactor using a peristaltic pump '). Prior to inoculating the production bioreactor, a flexible transfer line is employed to connect the bottom drain valve of the expansion bioreactor and the inoculation port of the production bioreactor. The transfer line and the tri-clamp connections are steam sterilized in place for 45±5 minutes. After the line cools down, the valves are opened and the expansion bioreactor is pressurized to transfer the culture into the production bioreactor). At harvest, the tangential-flow microfiltration clarified conditioned medium is transported by a peristaltic pump through a 0.2 µm filter into the harvest tank. The 0.2 µm filter is located at the head plate of the harvest tank and is steam sterilized before use.

Fermentation Programmable Logic Controllers Systems

The medium and harvest tanks are equipped with programmable logic controller (PLC)-based temperature and agitation speed controls. PLCs are also employed in the bioreactor systems to control temperature, pH, dissolved oxygen, agitation, and gas flows, as well as SIP and CIP functions. A personal computer is used to service the bioreactor systems for real-time system monitoring, data acquisition, alarm documentation, and setpoint down-loading. The tangential-flow microfiltration unit is equipped with a PLC for transmembrane pressure control. The validation of these systems are detailed in Sections 15.7 and 15.8.

Fermentation Biological Waste Handling

All liquid waste (cell-containing and cell-free) in the cell culture area is discharged through floor drains into a validated sodium hypochlorite-based waste treatment system. The chemical inactivation is carried out by maintaining the available chlorine concentration at greater than 5 parts per million (ppm) for at least 30 minutes. The inactivated solution is then neutralized prior to release into the sanitary sewer. All solid waste is collected in biological hazard bags (SOP DV-7002), and removed from the facility by a licensed medical waste management contractor. In the event of a bioreactor contamination, the broth is discharged into the waste treatment system and the bioreactor is cleaned either manually (expansion bioreactor) or by CIP (production bioreactor), and steam-sterilized
An investigation is launched to identify the microbial contaminant(s) and the